Attorney Docket No. 5051-653 In re: Jason C. H. Shih et al.

Application No.: 10/661,172 Filed: September 13, 2003

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## In the Specification:

Please amend the specification as follows:

At page 5, please replace the paragraph starting at the seventh line from the bottom of the page with the following paragraph:

Newly constructed plasmids (pNKER1, PNKER2 and pNKER43) described above were further transformed into *B. subtilis licheniformis* DB104. Transformation of *B. subtilis licheniformis* DB104 was carried out by the competence cell method as previously described (Lin et. al, 1997). The fidelity of the *ker*A insert in vectors was verified by restriction enzyme digestion analysis.

At page 9, please replace the paragraph starting at the sixth line from the bottom of the page and ending at the third line from the top of page 10 with the following paragraph:

In the present invention, stable *B. licheniformis* strains carrying multiple integrated *ker*A in chromosome were constructed to overproduce keratinase. Different gene copy number ranging from one to eight (data not shown) in the chromosome was successfully isolated by incorporating certain degrees of neomycin in the selective medium. Compared to the *B. subtilis* expression system, stable integrants producing higher enzyme activity were developed. Unlike the plasmid-containing expression system in *B. subtilis* previously developed, the new chromosomal integration of *ker*A in *B. licheniformis* avoided the segregational and structural instability common to replicative plasmids (Bron and Luxen, 1985; Harington et al., 1988; Primrose and Ehrlich, 1981).

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## In the Claims:

This listing of the claims will replace all prior versions and listings of the claims in the application:

- 1. (Currently Amended) A method of making a keratinase, comprising:
- (a) culturing a recombinant Bacillus in a medium, said recombinant Bacillus selected from the group consisting of Bacillus licheniformis and Bacillus subtilis and having at least one heterologous kerA coding sequence inserted into the chromosome thereof, with said recombinant Bacillus producing greater quantities of keratinase than a corresponding wild-type Bacillus that does not have said at least one heterologous kerA coding sequence inserted into the genome thereof; and then
  - (b) collecting isolating said keratinase from said medium.
- 2. (Currently Amended) The method of claim 1, wherein said medium comprises not more than 3% keratinase protein substrate.
- 3. (Currently Amended) The method of claim 1, wherein said medium comprises 1% sey soy flour and 1% feather meal.
  - 4. (Canceled)
- 5. (Original) The method of claim 1, wherein said *Bacillus* is *Bacillus* licheniformis.
- 6. (Previously Presented) The method of claim 1, wherein said kerA coding sequence is a Bacillus licheniformis or Bacillus subtilis kerA coding sequence.
- 7. (Previously Presented) The method of claim 1, wherein said kerA coding sequence is a Bacillus licheniformis kerA coding sequence.
- 8. (Original) The method of claim 1, wherein said corresponding wild-type *Bacillus* is *Bacillus licheniformis* PWD-1.

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9. (Currently Amended) The method of claim 1, said recombinant *Bacillus* 

having a plurality of said heterologous kerA coding sequence sequences inserted into the

chromosome thereof.

10. (Currently Amended) The method of claim 1, said recombinant Bacillus

having from 3 to 5 of said heterologous kerA coding sequences inserted into the

chromosome thereof.

11. (Currently Amended) The method of claim 1, wherein said recombinant

Bacillus is a keratinase-deficient Bacillus.

12. (Previously Presented) The method of claim 1, wherein said kerA coding

sequence is operatively associated with a constitutive promoter.

13. (Previously Presented) The method of claim 1, wherein said kerA coding

sequence is operatively associated with a P43 promoter.

14.-30. (Canceled)

31. (New) A method of making a keratinase, comprising:

(a) culturing a recombinant Bacillus in a medium, said recombinant Bacillus selected

from the group consisting of Bacillus licheniformis and Bacillus subtilis and having at least

one Bacillus licheniformis kerA coding sequence inserted into the chromosome thereof, with

said recombinant Bacillus producing greater quantities of keratinase than a corresponding

wild-type Bacillus that does not have said at least one Bacillus licheniformis kerA coding

sequence inserted into the genome thereof; and then

(b) isolating said keratinase from said medium, wherein said medium comprises not

more than 3% keratinase protein substrate.